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(54) Title: CONTROL OF GENE EXPRESSION BY IONIZING RADIATION

(57) Abstract

This invention relates to genetic constructs which comprise an enhancer-promoter region which is responsive to radiation, and at least one structural gene whose expression is controlled by the enhancer-promoter. This invention also relates to methods of destroying, altering, or inactivating cells in target tissue by delivering the genetic constructs to the cells of the tissues and inducing expression of the structural gene or genes in the construct by exposing the tissues to ionizing radiation. This invention is useful for treating patients with cancer, clotting disorders, myocardial infarction, and other diseases for which target tissues can be identified and for which gene expression of the construct within the target tissues can alleviate the disease or disorder.

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CONTROL OF GENE EXPRESSION BY IONIZING RADIATION

This invention relates to methods of controlling
gene expression by radiation responsive genetic
5   constructs. This invention also relates to methods and
compositions for destroying, altering, or inactivating
target tissues. These tissues may be disease-related,
for example, tumors, or blood clots, or they may have a
metabolic deficiency or abnormality. An aspect of this
10 invention is to deliver radiation responsive genetic
constructs to target tissues and to activate the genes in
said constructs by exposing the tissues to external
ionizing radiation.

15 Certain genes may play a role in the cellular
response to stress or DNA-damaging agents. For example,
metallothionein I and II, collagenase, and plasminogen
activator are induced after UV irradiation (Angel, et
al., 1986; 1987; Fornace, et al., 1988a and b; Miskin, et
20 al., 1981). B2 polymerase III transcripts are increased
following treatment by heat shock (Fornace, et al., 1986;
1989a). Furthermore, although the level of DNA
polymerase β mRNA is increased after treatment with DNA-
damaging agents, this transcript is unchanged following
25 irradiation, suggesting that specific DNA-damaging agents
differentially regulate gene expression (Fornace, et al.,
1989b). Protooncogene c-*fos* RNA levels are elevated
following treatment by UV, heat shock, or chemical
carcinogens (Andrews, et al., 1987; Hollander, et al.,
30 1989a). In this regard, the relative rates of *fos*
transcription during heat shock are unchanged, suggesting
that this stress increased c-*fos* RNA through
posttranscriptional mechanisms (Hollander, et al.,
1989b).

35

Investigations of the cytotoxic effects of ionizing

radiation has focused on the repair of DNA damage or the modification of radiation lethality by hypoxia (Banura, et al., 1976; Moulder, et al., 1984). In prokaryotes and lower eukaryotes, ionizing radiation has been shown to induce expression of several DNA repair genes (Little, et al., 1982); however, induction of gene expression by ionizing radiation has not been described in mammalian cells. DNA-damaging agents other than x-rays induce expression of a variety of genes in higher eukaryotes (Fornace, et al., 1988, 1989; Miskin, et al., 1981).

What is known about the effects of ionizing radiation is that DNA damage and cell killing result. In many examples, the effects are proportional to the dose rate. Ionizing radiation has been postulated to induce multiple biological effects by direct interaction with DNA or through the formation of free radical species leading to DNA damage (Hall, 1988). These effects include gene mutations, malignant transformation, and cell killing. Although ionizing radiation has been demonstrated to induce expression of certain DNA repair genes in some prokaryotic and lower eukaryotic cells, little is known about the effects of ionizing radiation on the regulation of mammalian gene expression (Borek, 1985). Several studies have described changes in the pattern of protein synthesis observed after irradiation of mammalian cells. For example, ionizing radiation treatment of human malignant melanoma cells is associated with induction of several unidentified proteins (Boothman, et al., 1989). Synthesis of cyclin and coregulated polypeptides is suppressed by ionizing radiation in rat REF52 cells but not in oncogene-transformed REF52 cell lines (Lambert and Borek, 1988). Other studies have demonstrated that certain growth factors or cytokines may be involved in x-ray-induced DNA damage. In this regard, platelet-derived growth factor

is released from endothelial cells after irradiation
(Witte, et al., 1989).

5   Initiation of mRNA synthesis by DNA is a critical
control point in the regulation of cellular processes and
depends on bindings of certain transcriptional regulatory
factors to specific DNA sequences. However, little is
known about the regulation of transcriptional control by
ionizing radiation exposure in eukaryotic cells. The
10   effects of ionizing radiation on posttranscriptional
regulation of mammalian gene expression are also unknown.

Many diseases, conditions, and metabolic
deficiencies would benefit from destruction, alteration,
15   or inactivation of affected cells, or by replacement of a
missing or abnormal gene product. In certain situations,
the affected cells are focused in a recognizable tissue.
Current methods of therapy which attempt to seek and
destroy those tissues, or to deliver necessary gene
20   products to them, have serious limitations. For some
diseases, e.g., cancer, ionizing radiation is useful as a
therapy. Methods to enhance the radition, thereby
reducing the necessary dose, would greatly benefit cancer
patients. Therefore, methods and compositions were
25   sought to enhance radiation effects by investigating
effects of radiation on gene expression. A goal was to
provide new types of therapy using radiation, and to
explore other uses of radiation.

30   In this invention, control exerted over gene
expression by a promoter-enhancer region, which is
responsive to ionizing radiation, is used as a switch to
selectively introduce gene products to distinct tissue
targets, providing opportunities for therapeutic
35   destruction, alteration, or inactivation of cells in
target tissues. These promoter-enhancer regions control

gene expression through application of a radiation
trigger.

More particularly, this invention relates to methods
and compositions for treating diseases and conditions for
which destruction, alteration or inactivation of cells in
affected tissues would alleviate the disease or
condition. The methods comprise delivering a genetic
construct to cells of the host tissue and subsequently
exposing the tissue to ionizing radiation. A region of
the genetic construct is capable of being induced by
ionizing radiation. Exposing the tissue to ionizing
radiation, therefore, induces the expression of the
genetic construct. The gene product is then capable of
destroying, altering, or inactivating the cells in the
tissue. The gene product chosen for treatment of factor
deficiencies or abnormalities, is one that provides the
normal n factor.

An illustrative embodiment of the genetic construct
comprises a combination of a radiation responsive
enhancer-promoter region and a region comprising at least
one structural gene. The enhancer-promoter region drives
the expression of a structural gene in the form of a
reporter-effector gene appropriate for the disease or
condition in the host.

The general composition of the construct comprises a
radiation inducible promoter-enhancer region and a
structural gene region. In an illustrative embodiment,
the promoter is 5' to the structural gene region. In
this embodiment, amplification of the final response does
not occur. Rather there is a direct correlation between
regulation of the radiation sensitive region and the
structural gene. The inducible region is turned on by
radiation exposure, but will turn off at some point after

the radiation exposure ceases. Expression of the
structural gene region is limited by exposure time and
the inherent quantitative limits of the expression
region.

5

In a preferred embodiment, to amplify the expression
of the gene construct and to extend expression beyond
exposure time, a cascade of promoters and expressing
genes are contemplated, for example, two plasmids. The
10   first plasmid comprises the radiation sensitive promoter
5' of an appropriate transcription factor. In an
embodiment of a transcription factor, the first plasmid
comprises a powerful activation domain, for example, that
obtained from the herpes virus VP16. This domain
15   contains many negatively charged residues. A chimeric
protein is contemplated in this embodiment comprising the
VP16 activation domain and a DNA binding domain of a
known protein, for example, the lac repressor. The
chimeric protein/gene construct (a fusion gene) is driven
20   from a radiation sensitive promoter.

The second plasmid construct in the preferred
embodiment comprises several binding sites for the lac
repressor DNA binding domain. These binding sites are
25   placed upstream of a reporter-effector gene, for example,
TNF. Alternatively, the two plasmids described above
could be merged into one construct.

The use of a cascade of promoters and two expressing
30   genes as the genetic construct has several advantages:
(1)   the promoter does not have to provide strong
activation because amplification of the initial
radiation sensitive promoter effect is provided
through action of the subsequent genetic
35   cascade;
(2)   several genes may be included in the construct

to provide more complex or more extensive
action. In an illustrative embodiment, several
toxin producing genes may be placed 3' of the
appropriate DNA binding sites. An embodiment
of a multiple gene construct comprises the DNA
binding domain of the lac repressor followed by
several genes which produce various regulators
of cell growth; and

(3) the effect due to the initial ionizing
radiation may be temporarily prolonged; that
is, if the half-life of the chimeric lac
repressor protein were long, for example, hours
or day, compared to the radiation exposure time
during which promoter RNA is released, the
effect of the genetic construct on the cell is
prolonged.

The genetic construct of this invention is
incorporated into the cells of a target tissue by any
method which incorporates the construct without
inhibiting its desired expression and control over that
expression by radiation. These methods comprise
electroporation, lipofection, or retroviral methodology.

Retroviruses used to deliver the constructs to the
host target tissues generally are viruses in which the 3'
LTR (linear transfer region) has been inactivated. That
is, these are enhancerless 3'LTR's, often referred to as
SIN (self-inactivating viruses) because after productive
infection into the host cell, the 3'LTR is transferred to
the 5' end and both viral LTR's are inactive with respect
to transcriptional activity. A use of these viruses well
known to those skilled in the art is to clone genes for
which the regulatory elements of the cloned gene are
inserted in the space between the two LTR's. An
advantage of a viral infection system is that it allows

for a very high level of infection into the appropriate
recipient cell, e.g., LAK cells.

For purposes of this invention, a radiation
5   responsive enhancer-promoter which is 5' of the
appropriate structural gene region, for example, a
lymphokyne gene, or a transcriptional activator, may be
cloned into the virus.

10   The constructs are delivered into a host by any
method that causes the constructs to reach the cells of
the target tissue, while preserving the characteristics
of the construct used in this invention. These methods
comprise delivering the construct by intravenous
15   injection, injection directly into a target tissue, or
incorporation into cells which have been removed from the
host. In the latter case, after *in vitro* incorporation
of the constructs into the recipient cells, the cells
containing the construct are reintroduced into the host.
20   Depending on the type of recipient cell, the distribution
of the cells in the host will vary – in some cases being
focused to a specific area, for example, where cells are
directed to a tumor or clot, in other cases diffusing
through an entire system such as the bone marrow. Even
25   when the cells carrying the genetic construct have
dispersed over a wide area of the host, focusing the
desired action of the construct on a target tissue can be
provided by directing the ionizing radiation used to
switch on the construct, to a limited area. Only the
30   cells within the beam will react and cause expression of
the construct genes.

Another method of focusing the genetic action of the
construct, or homing it into particular body regions, is
35   to tag the construct with a radioisotope or other label
and determine when the construct bearing cells have

reached the target tissue by detecting the label geographically. The radiation is turned on when the construct reaches the target, and directed to the labelled direction.

The type of recipient cells used to incorporate the radiation inducible genetic constructs are selected based on the objective of the treatment. In an exemplary embodiment, LAK cells are used for patients in which tumor-directed attack is the main objective. In another embodiment, endothelial cells are used to deliver genes for gene therapy, for example, to treat genetically abnormal fetuses with a metabolic deficiency or abnormality. Cells derived from peripheral blood are also suitable recipient cells.

In an exemplary embodiment of the genetic construct, there are several steps leading to expression of the structural gene in the host tissues. In these constructs, there is a radiation sensitive promoter which causes (drives) the expression of a transcription factor. The transcription factor activates a reporter construct which includes an effector appropriate for the disease or condition of the host. The expression production of the effector gene interacts in a therapeutic fashion with the diseased, deficient or abnormal cells without a target tissue.

In an exemplary embodiment, toxins which are capable of killing tumor cells are put into LAK cells or other cellular/molecular vehicles by incorporating into the cells a vector comprising a radiation inducible or responsive promoter-enhancer region and a structural gene region. Examples of a radiation responsive promoter-enhancer region comprise that derived from, for example, c-jun or TNF-α. Examples of structural genes comprise

those expressed as tumor necrosis factor (TNF), ricin, or
various growth factors including, but not limited to, IL-
1-6, PDGF (platelet derived growth factor) or FGF
(fibroblast growth factor). Diseases for which this
5   embodiment of a construct is useful comprise cancers.
Types of cancers which would benefit from this form of
treatment comprise solid and hematologic malignancies.
Specific cancers include head and neck adenocarcinomas.

10   An embodiment of genetic construct comprises a
radiation sensitive promoter coupled to an appropriate
reporter, for example, β-galactosidase. The construct is
transferred to a recipient cell. In general, many
recipient cells are prepared in this fashion. The
15   recipient cells are then introduced into a mammal. In an
illustrative example, endothelial cells are used as the
recipient cells. These cells are then transplanted into
an appropriate blood vessel in which the action of the
construct within the cells is desired. Radiation is
20   delivered to an area of the body including that blood
vessel. Expression of the β-galactosidase is monitored
by chromogenic assays such as Xgal.

          An embodiment of a structural gene which acts as a
25   reporter-effector gene comprises that which is expressed
as the tumor necrosis factor (TNF). Increased TNF-α
production by human sarcomas after x-irradiation is
evidence for the direct cytotoxic effects of this
polypeptide on human tumor cells (Sugarman, 1985; Old,
30   1985). The intracellular production of TNF-α within
irradiated tumor cells results in lethality to the cell
after x-ray exposure that is greater than the lethality
produced by the direct effects of ionizing radiation
alone.
35
          The additive and synergistic effects, the latter

occurring if TNF is provided before radiation, of TNF-α
on tumor killing by radiation supports potential
applications for the use of TNF-α in clinical
radiotherapy. TNF-α potentiates the cellular immune
5   response (Bevelacqua, et al., 1989; Sersa, et al., 1988).
In vivo studies have shown that TNF-α enhances tumor
control by x-rays in mice with implanted syngeneic tumors
by the augmentation of the host's immune system (Sersa,
et al., 1988). Therefore, TNF-α may reverse immune
10   suppression, which often accompanies radiotherapy. TNF-α
also causes proliferation of fibroblasts and endothelial
destruction, suggesting that TNF-α production by tumors
may be one component responsible for the late radiation
effects in surrounding normal tissue. Turning on this
15   gene within a genetic construct by radiation allows
directed attack on diseased tissues.

      In addition to killing tumor cells by treatment with
TNF, a goal is to protect normal tissues adjacent to the
20   target tissue from radiation effects and deleterious
action of various cytotoxins during cancer or other
therapy. Solid and hemologic malignancies and aplastic
anemia, are conditions for which this is a concern.
Genes in the structural region of the genetic construct
25   of this invention that are appropriate for this
protective goal, include lymphokines, GCSF, CMSF, and
erythropoietin.

      The goal of cancer treatment is not only to kill
30   cells at a specific target, but to inhibit metastasis.
For this purpose, one of the genes appropriate for
inclusion in the genetic construct is NM23.

      Prevention of secondary malignancies which are and
35   unfortunate side effect of standard radiotherapy and
chemotherapy, is assisted by treatment with a construct

comprising tumor suppressor genes.

This invention has uses in diseases and conditions
other than cancer. For patients with clotting disorders,
Factor VIII or other factors necessary for the complex
process of clot formation, may be introduced into cells
deficient for the missing factor.

Conversely, in conditions such as myocardial
infarction, central nervous system or peripheral
thrombosis, anticlotting factors introduced via the
genetic constructs of this invention, are used to
dissolve the clots. Embodiments of the expression
products of such genes include streptokinase and
urokinase.

Other categories of diseases or conditions for which
there is a deficiency due to either a genetic or
environmental factor, include the hemoglobinopathies such
as sickle cell anemia, for which genes producing normal
hemoglobin are included in the treatment construct;
neurodegenerative diseases such as Alzheimer's disease
for which genes expressed as nerve growth factors are
included in the construct; and diabetes, for which
insulin producing genes may be included in the construct.

Genetic diseases caused by defects in the genetic
pathways effecting DNA repair, e.g., ataxia
telangiectasia, xeroderma pigmentosum, are treated by the
introduction of genes such as ERCC-1 or XRCC-1.

Although the practice of this invention requires
exposure to radiation, an agent which in itself may
adversely affect cells, the dose is relatively low,
administered for brief periods of time, and focused. For
many of the diseases and conditions for which this

invention is appropriate, radiation treatment is standard, and practice of this invention will reduce the necessary dose, which reduces risk of the radiation treatment per se. For diseases which usually do not

5   require radiation, use of radiation in the methods described in this invention will replace another therapy. Decision on use of this invention will be based on a risk/benefit analysis.

Definitions

10   Effector Gene - a gene whose expression product produces the desired effect in the recipient cells and target tissues.

Enhancer Gene or Element - a *cis-* acting nucleic acid

15   sequence that increases the ulitization of some eukaryotic promoters, and can function in either orientation and in any location (upstream or downstream) relative to the promoter.

LAK Cells - lymphocyte activated killer cells.

20   Promoter - a region of DNA involved in binding RNA polymerase to initiate transcription.

Reporter Gene - a gene whose expression product is readily detectable and serves as a marker for the expression of induction.

25   Structural Gene - a gene coding for a protein with an effector function. This protein might be an enzyme, toxin, ligand for a specific receptor, receptor, nucleic acid binding protein or antigen. The protein could also serve as a reporter to monitor induction by ionizing

30   radiation. The gene coding for these proteins could be derived from eukaryotes or prokaryotes.

Other objects and advantages of the invention will become apparent upon reading the following detailed

35   description and upon reference to the drawings in which:

FIG. 1. A schematic drawing of the basic genetic construct comprising a radiation sensitive promoter driving an effector gene.

5   FIG. 2. A schematic drawing of a more complex genetic construct than that shown in FIG. 1, comprising an "amplification system."

FIG. 3. A schematic drawing comprising the basic
10   system of a retroviral mode of infection of a genetic construct into a cell.

FIG. 4. Effects of irradiation on TNF-α gene expression.

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FIG. 5. Influence of TNF-α on radiation lethality of TNF-α-producing human sarcomas and TNF-α-nonproducing human tumor cells.

20   FIG. 6. Effects of ionizing radiation on c-jun RNA levels in human HL-60 cells.

FIG. 7. Effects of ionizing radiation on c-jun RNA levels in U-937 cells and in human AG-1522 diploid
25   fibroblasts.

FIG. 8. Effects of ionizing radiation on rates of c-jun gene transcription.

30   FIG. 9. Effects of cycloheximide on c-jun mRNA levels in ionizing radiation-treated HL-60 cells.

FIG. 10. Effects of ionizing radiation on C-fos and jun-B mRNA levels in HL-60 cells.

35

FIG. 11. Effects of dose rate on the induction of c-*jun* expression by ionizing radiation.

While the invention is susceptible to various
5   modifications and alternative forms, a specific
embodiment thereof has been shown by way of example in
the drawings and will herein be described in detail. It
should be understood, however, that it is not intended to
limit the invention to the particular forms disclosed,
10 but on the contrary, the intention is to cover all
modifications, equivalents, and alternatives falling
within the spirit and scope of the invention as defined
by the appended claims.

15   This invention relates to methods and compositions
of controlling expression of a gene by exposure of a
construct, including the gene, to ionizing radiation.
The genes to be controlled are preferably incorporated
within a genetic construct which includes a region which
20 is sensitive to ionizing radiation. A schematic diagram
of such a construct is shown in FIG. 1 wherein an
enhancer-promoter region 10 of a radiation response gene,
e.g., c-*jun*, drives 16 the expression of a structural
gene, e.g., a reporter-effector gene such as TNF 14. The
25 product of the structural gene expression is then capable
of acting on a cell which has incorporated it, to produce
a desired effect on the cell.

A more complex genetic construct is shown
30 schematically in FIG. 2. In FIG. 2A, a region 20
comprising an enhancer-promoter of a radiation responsive
gene, is coupled to, and drives 28 the expression of, a
DNA binding domain 26, e.g., of a LAC repressor gene, and
a gene 24 producing a transcription factor, e.g., from
35 VP16. The chimeric protein resulting from the expression
of that fusion gene, 40, 42 is capable of binding to a

DNA sequence 30 illustrated in FIG. 2B. Binding of this
sequence by the transcription factor 40, 42 activates 38
a structural gene 36, e.g., a reporter-effector gene such
as TNF. A "minimal promoter" 32 containing CCAAT and the

5 TATA boxes, e.g., from the c-*fos* oncogene, is placed
between the binding sequence 30 and the genes 36 to be
expressed. The gene product 34 is capable of acting on a
cell which has incorporated the genetic constructs, to
produce a desired effect.

10

An example showing details of the multiple gene form
of genetic construct is shown in FIG. 2. This figure is
predicated on strong induction of the c-*jun* gene in
various different cell types by ionizing radiation at a

15 transcriptional level. A large piece of 5' genomic
sequence from the *jun* gene is ligated to an appropriate
reporter such as β-galactosidase. Such a construct is
then transfected into a recipient cell and checked for
radiation responsiveness. Various truncations of this

20 initial large 5' piece may be used.

Methods of incorporating constructs into recipient
cells comprise electroporation, lipofection, and viral
infection. This latter method comprises a SIN (self-

25 inactivating virus) with two LTR's 50, 56. Nestled
between the LTR's is a genetic construct comprising a
radiation sensitive element 52 and a structural gene
region 54. A U3 enhancer deletion is shown at 58.

30   Examples of elements used for the constructs follow.

Radiation Regulates TNF-α Expression

Combinations of tumor necrosis factor α (TNF-α), a
35 polypeptide mediator of the cellular immune response with
pleiotropic activity, and radiation produce synergistic

effects and are useful for clinical cancer therapy. TNF-
α acts directly on vascular endothelium to increase the
adhesion of leukocytes during the inflammatory process
(Bevelacqua, et al., 1989). This *in vivo* response to

5   TNF-α was suggested to be responsible for hemorrhagic
necrosis and regression of transplantable mouse and human
tumors (Carswell, 1975). TNF-α also has a direct effect
on human cancer cell lines *in vitro*, resulting in cell
death and growth inhibition (Sugarman, et al., 1985; Old,

10   1985). The cytotoxic effect of TNF-α correlates with
free-radical formation, DNA fragmentation, and
microtubule destruction (Matthews, et al., 1988; Rubin,
et al., 1988; Scanlon, et al., 1989; Yamauchi, et al.,
1989; Matthews, et al., 1987; Neale, et al., 1988). Cell

15   lines that are resistant to oxidative damage by TNF-α
also have elevated free-radical buffering capacity
(Zimmerman, et al., 1989; Wong, et al., 1988).

TNF-α causes hydroxyl radical production in cells

20   sensitive to killing by TNF-α (Matthews, et al., 1987).
Cell lines sensitive to the oxidative damage produced by
TNF-α have diminished radical-buffering capacity after
TNF-α is added (Yamauchi, et al., 1989). Lower levels of
hydroxyl radicals have been measured in cells resistant

25   to TNF-α cytotoxicity when compared with cells sensitive
to TNF-α killing (Matthews, et al., 1987).

Tumor necrosis factor α is increased after treatment
with x-rays in certain human sarcoma cells. The increase

30   in TNF-α mRNA is accompanied by the increased production
of TNF-α protein.

The induction of a cytotoxic protein by exposure of
cells containing the TNF gene to x-rays was suspected

35   when medium decanted from irradiated cultures of some
human sarcoma cell lines was found to be cytotoxic to

those cells as well as to other tumor cell lines. The
level of TNF-α in the irradiated tumor cultures was
elevated over that of nonirradiated cells when analyzed
by the ELISA technique (Saribon, et al., 1988).

5   Subsequent investigations showed that elevated TNF-α
protein after irradiation potentiates x-ray killing of
cells by an unusual previously undescribed mechanism (see
Example 1).

10   FIG. 4 illustrates the effects of irradiation on
TNF-α gene expression. RNA from untreated cells
(control) and irradiated cells was size-fractionated and
hybridized to ^{32}P-labeled TNF-α cDNA (STSAR-13) and PE4
plasmid containing TNF-α cDNA (STSAR-48). Autoradiograms

15   showed increased expression of TNF-α mRNA 3 hr after
irradiation in cell line STSAR-13 and at 6 hr in cell
line STSAR-48. 7S RNA was hybridized to show the pattern
for equally loaded lanes. The conclusion from these
results is that there is increased TNF-α gene expression

20   after radiation.

       The next question was what the effects of TNF-α and
radiation would be on cell killing. FIG. 5 exhibits the
influence of TNF-α on radiation lethality of TNF-α-

25   producing human sarcomas and TNF-α-nonproducing human
tumor cells. The solid lines indicate the effects of
radiation alone, and the dashed lines indicate the
effects of both TNF-α and irradiation. Representative
survival data for cell line STSAR-33 are shown in the

30   graph to the left, A. The lower dashed line represents
survival of cells with TNF-α at 1000 units/ml, corrected
for a plating efficiency (PE) of 30%. The survival of
human epithelial tumor cells (SQ-20B) irradiated with
TNF-α (10 units/ml and 1000 units/ml) is shown in the

35   middle graph, B. Survival data for SQ-20B show an
additive effect of TNF-α (1000 units/ml). Survivals with

TNF-α are corrected for 85% killing with TNF-α alone. Radiation survival data for HNSCC-68 is shown in the graph to the right, C. A nonlethal dose of TNF-α (10 units/ml) was added 24 hr before irradiation.

As can be seen from these results and from information discussed in EXAMPLE 1, the tumor necrosis factor α is increased after treatment with x-rays. Both mRNA and TNF-α proteins were increased.

Although DNA-damaging agents other than ionizing radiation have been observed to induce expression of variety of prokaryotic and mammalian genes, the TNF-α gene is the first mammalian gene found to have increased expression after exposure to ionizing radiation. This gene is not categorized as a DNA repair gene.

To determine the mechanisms responsible for regulation of c-jun gene expression by ionizing radiation, run-on transcriptional assays were performed in isolated nuclei. The action gene was constitutively transcribed in untreated HL-60 cells as a positive control (FIG. 8).

Negative control was provided by the β-globin gene transcript. As shown in FIG. 8, a low level of c-jun transcription was detectable in HL-60 untreated by radiation. Dramatic increased transcription (7.2 fold) occurred after exposure to ionizing radiation. The conclusion from this study was that ionizing radiation induced c-jun expression, at least in part by a transcriptional mechanism.

FIG. 9 illustrates the effects of cycloheximide on c-jun mRNA levels in ionizing radiation treated HL-60 cells. The columns headed XRT shows expression of mRNA

after 20 Gy radiation exposure of the cells. In the
columns CHX, cycloheximide has been added. The additive
effects of CHX and CHX/XRT are a 3.6 fold increased
expression compared to XRT alone.

5

Effects of cycloheximide on c-*jun* mRNA levels in
ionizing radiation-treated HL-60 cells. HL-60 cells were
treated with 20 Gy of ionizing radiation (XRT) and/or 5
μg of cycloheximide (CHX) per ml. Total cellular RNA (20
10   μg per lane) was isolated after 1, 3 and 6 h and analyzed
by hybridization to the ^{32}P-labeled c-*jun* or actin probe.

FIG. 10. Effects of ionizing radiation on C-*fos* and
jun-B mRNA levels in HL-60 cells. (A) HL-60 cells were
15   treated with varying doses of ionizing radiation (XRT) or
32 nM 12-*O*-tetradecanoylphorbol 13-acetate (TPA; positive
control) for 3 h. Total cellular RNA (20 μg) was
hybridized to the ^{32}P-labeled c-*fos* probe. (B) HL-60
cells were treated with 20 Gy of ionizing radiation.
20   Total cellular RNA (20 μg per lane) was isolated at the
indicated times and analyzed by hybridization to the ^{32}P-
labeled *jun-B* probe.

FIG. 11. Effects of dose rate on the induction of
25   c-*jun* expression by ionizing radiation. HL-60 cells were
treated with 10 or 20 Gy of ionizing radiation at the
indicated dose rates. After 3 h, total cellular RNA (20
μg) was isolated and hybridized to the ^{32}P-labelled c-*jun*
probe.

30

Targeting Tissues for Incorporation of a
Genetic Construct Responsive to Ionizing Radiation

Depending on the application in question, the
35   recipient cells are targeted in various ways. In an
exemplary embodiment, LAK cells which tend to home in on

the tumor site in question with some degree of preference though as is well known, they will also distribute themselves in the body in other locations, may be used to target tumors. Indeed, one of the most important

5   advantages of the radiation inducible system is that only those LAK cells, which are in the radiation field will be activated and will have their exogenously introduced lymphokine genes activated. Thus, for the case of LAK cells, there is no particular need for any further

10   targeting. In other applications, the appropriate cells in question have had appropriate genes from monoclonal antibodies introduced in them or appropriate antibodies expressed on their cell surface by other means such as by cell fusion. These monoclonal antibodies, for example,

15   are targeted towards specific cells in the body and thus allow the recipient cells to home in on that particular region so that then radiation could be used for the activation of the appropriate toxins within them. This enables local delivery of the "drug," wherein the "drug"

20   is defined as the expression product of the genes within the radiation responsive genetic construct. Illustrative embodiments of types of radiation inducible constructs and their applications are presented in Table 1 and EXAMPLE 4.

25

TABLE 1: ILLUSTRATIVE EMBODIMENTS OF TYPES OF RADIATION INDUCIBLE GENETIC CONSTRUCTS AND THEIR USES

Action of Expression Products of Genes in the Construct	Examples of Structural Genes Used in the Construct	Applications to Diseases, Conditions and Tissues
Kill tumor cells	Toxins TNF Growth Factors (IL-1-6 PDGF, FGF)	Solid and Hematologic Malignancies
Protect normal tissues from radiation and other cytotoxins during cancer therapy	Lymphokines GCSF, CMCSF Erythropoietin	Solid and Hematologic Malignancies, Aplastic Anemic
Inhibit Metastasis	NM23	Cancer Metastasis
Tumor Suppressor Gene Products	Rb p53	Prevention of Malignancy Following Standard Radiotherapy and Chemotherapy
Radiosensitization Chemosensitization (enhance routine treatment effects)	TNF	Solid and Hematologic Malignancies
Correct Defects in Clotting Factors	Factor 8	Clotting Disorders
Introduce Anticlotting Factors	Streptokinase Urokinase	Myocardial Infarction, CNS Thrombosis, Pheripheral Thrombosis
Correct Defects Characterizing Hemoglobinopathy	Normal Hemoglobin	Sickle Cell Anemia
Correct Deficienceis Leading to Neurodegenerative Disease	Nerve Growth Factor	Alzheimer's Disease
Provide Treatment Component for Diabetes	Insulin	Diabetes

Action of Expression Products of Genes in the Construct	Examples of Structural Genes Used in the Construct	Applications to Diseases, Conditions and Tissues
Disease of DNA Repair Abnormalities	ERCC-1, XRCC-1	Ataxia Telangiectasia Xeroderma Pigmentosum

EXAMPLES

EXAMPLE 1
Increased Tumor Necrosis Factor α mRNA
After Cellular Exposure to Ionizing Radiation

A. Protein Products

To investigate TNF-α protein production after x-irradiation, the levels of TNF-α in the medium of human tumor cell lines and fibroblasts were quantified by the ELISA technique (Saribon, et al., 1988) before and after exposure to 500-cGy x-rays (Table 1). Five of 13 human bone and soft tissue sarcoma cell lines (STSAR-5, -13, -33, -43, and -48) released TNF-α into the medium after irradiation, whereas TNF-α levels were not elevated in supernatant from normal human fibroblast cell lines (GM-1522 and NHF-235) and four human epithelial tumor cell lines (HN-SCC-68, SCC-61, SCC-25, and SQ-20B) after exposure to radiation. The assay accurately measures TNF-α levels between 0.1 and 2.0 units per ml (2.3 x 10^6 units/mg) (Saribon, et al., 1988). Tumor cell line STSAR-13 produced undetectable amounts of TNF-α before x-irradiation and 0.35 units/ml after x-ray exposure. Cell lines STSAR-5 and -33 responded to x-irradiation with increases in TNF-α concentrations of >5- to 10-fold; however quantities above 2 units/ml exceeded the range of the assay (Saribon, et al., 1988). Cell lines STSAR-43 and -48 demonstrated increases in TNF-α of 1.5- to 3-fold (Table 1). TNF-α protein in the medium was first

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elevated at 20 hr after x-ray treatment, reached maximal
levels at 3 days, and remained elevated beyond 5 days.
Furthermore, supernatant from irradiated, but not control
STSAR-33, was cytotoxic to TNF-α-sensitive cell line SQ-
5   20B.

TABLE 2:   PRODUCTION OF TNF-A IN HUMAN SARCOMA CELL LINES

Cell Line	Origin	TNF-α level, units/ml,	
		Control	X-ray
STSAR-5	MFH	0.4	>2.0
STSAR-13	Liposarcoma	0.0	0.34
STSAR-33	Ewing sarcoma	0.17	>2.0
STSAR-43	Osteosarcoma	0.41	1.3
STSAR-48	Neurofibrosarcoma	0.28	0.43

    TNF-α levels were measured in medium from confluent
cell cultures (control) and in irradiated confluent cells
(x-ray). TNF-α levels increased as measured by the ELISA
technique. MFH, malignant fibrous histiocytoma.

    B.   RNA Analysis.

    · Increased levels of TNF-α mRNA were detected in the
TNF-α-producing sarcoma cell lines after irradiation
relative to unirradiated controls (FIG. 4). For example,
TNF-α transcripts were present in unirradiated STSAR-13
and -48 cell lines. TNF-α mRNA levels in cell line
STSAR-13 increased by >2.5-fold as measured by
densitometry 3 hr after exposure to 500 cGy and then
declined to baseline levels by 6 hr (FIG. 4). These
transcripts increased at 6 hr after irradiation in cell
line STSAR-48, thus indicating some heterogeneity between
cell lines in terms of the kinetics of TNG-α gene
expression (FIG. 4). In contrast, irradiation had no
detectable effect on 7S RNA levels (FIG. 4) or expression
of the polymerase β gene.

C.   Interaction Between TNF-α and X-Irradiation.

To investigate the influence of TNF-α on radiation-induced cytotoxicity in TNF-α-producing cell lines, recombinant human TNF-α was added to cultures before irradiation (FIG. 5). Recombinant human TNF-α (1000 units/ml) (2.3×10^6 units/mg) was cytotoxic to four of five TNF-α-producing sarcomas (STSAR-5, -13, -33, and -43). The plating efficiency (PE) was reduced by 60-90% at 1000 units/ml in these lines. Radiation-survival analysis of cell line STSAR-33 was performed with TNF-α (10 units/ml). The radiosensitivity (D_0), defined as the reciprocal of the terminal slope of the survival curves was 80.4 cGy for cell line STSAR-33. When TNF-α was added 20 hr before irradiation, the D_0 was 60.4 cGy. Surviving fractions were corrected for the reduced PE with TNF-α. Thus, the interaction between TNF-α and radiation in STSAR-33 cells was synergistic (Dewey, 1989). Sublethal concentrations of TNF-α (10 units/ml) enhanced killing by radiation in cell line STSAR-33, suggesting a radiosensitizing effect of TNF-α The surviving fraction of cell line STSAR-5 at 100-700 cGy was lower than expected by the independent killing of TNF-α and x-rays, although the D_0 values were similar. Thus, the interaction between TNF-α and radiation is additive (Dewey, 1979) in STSAR-5 cells. Cell lines STSAR-13 and STSAR-43 were independently killed with x-rays and TNF-α, and no interaction was observed.

To determine the possible interactions between TNF-α and x-rays in non-TNF-α producing cells, human epithelial tumor cells (SQ-20B and HNSCC-68) were irradiated 20 hr after TNF-α was added. These cell lines do not product TNF-α in response to ionizing radiation. TNF-α (1000 units/ml) was cytotoxic to SQ-20B and SCC-61 cells, reducing the PE by 60-80%. The radiation survival of SQ-20B cells with and without TNF-α is shown in FIG. 5. The

D_0 for cell line SQ-20B is 239 cGy. With TNF-α (1000
units/ml) added 24 hr before x-rays, the D_0 was 130.4 cGy.
Therefore, a synergistic interaction (Dewey, 1979)
between TNF-α and x-rays was demonstrated in this cell

5   line. TNF-α added after irradiation did not enhance cell
killing by radiation in cell lines SQ-20B. Nonlethal
concentrations of TNF-α (10 units/ml) resulted in
enhanced radiation killing in cell line HNSCC-68 (FIG.
5), providing evidence that TNF-α may sensitize some

10   epithelial as well as mesenchymal tumor cell lines to
radiation.

The following specific methods were used in Example
1.

15

Cell Lines. Methods of establishment of human
sarcoma and epithelial cell lines have been described
(Weichselbaum, et al., 1986; 1988). Culture medium for
epithelial tumor cells was 72.5% Dulbecco's modified

20   Eagle's medium/22.5% Ham's nutrient mixture F-12 [DMEM/F-
12 (3:1)]5% fetal bovine serum (FBS), transferrin at 5
μg/ml/10^{-10} M cholera toxin/1.8 x 10^{-4} M adenine,
hydrocortisone at 0.4 μg/ml/2 x 10^{-11} M triodo-L-
thyronine/penicillin at 100 units/ml/streptomycin at 100

25   μg/ml. Culture medium for sarcoma cells was DMEM/F-12
(3:1)/20% FBS, penicillin at 100 units/ml/streptomycin at
100 μg/ml.

TNF-α Protein Assay. Human sarcoma cells were

30   cultured as described above and grown to confluence. The
medium was analyzed for TNF-α 3 days after feeding and
again 1-3 days after irradiation. Thirteen established
human sarcoma cell lines were irradiated with 500-
centigray (cGy) x-rays with a 250-kV Maxitron generator

35   (Weichselbaum, et al., 1988). TNF-α was measured by
ELISA with two monoclonal antibodies that had distinct

epitopes for TNF-α protein (Saribon, et al., 1988); the
assay detects TNF-α from 0.1 to 2.0 units/ml.

RNA Isolation and Northern (RNA) Blot Analysis.

5   Total cellular RNA was isolated from cells by using the
guanidine thiocyanate-lithium chloride method (Cathala,
et al., 1983). RNA was size-fractionated by
formaldehyde-1% agarose gel electrophoresis, transferred
to nylon membranes (GeneScreenPlus, New England Nuclear),
10 hybridized as previously described to the 1.7-kilobase
(kb) BamHI fragment of the PE4 plasmid containing TNF-α
cDNA (19, 23), and autoradiographed for 16 days at -85·C
with intensifying screens. Northern blots were also
hybridized to 7S rRNA and β-polymerase plasmids as
15 described (Fornace, et al., 1989). Ethidium bromide
staining revealed equal amounts of RNA applied to each
lane. RNA blot hybridization of TNF-α was analyzed after
cellular irradiation with 500 cGy. Cells were washed
with cold phosphate-buffered saline and placed in ice at
20 each time interval. RNA was isolated at 3, 6, and 12 hr
after irradiation.

Treatment of Cells with X-Irradiation and TNF-α.

Exponentially growing cells were irradiated by using a
25 250-kV x-ray generator. The colony-forming assay was
used to determine cell survival (Weichselbaum, et al.,
1988). The multitarget model survival curves were fit to
a single-hit multitarget model $[S = 1 - (-e^{-D|D0})^n]$.
Concentrations of recombinant human TNF-α (10 units/ml)
30 $(2:3 \times 10^6$ units/mg) and (1000 units/ml) (Asahi Chemical,
New York) were added 24 hr before irradiation.

EXAMPLE 2
Increased c-jun Expression After
Exposure to Ionizing Radiation

35

The following methods were used in this example.

Radiation Regulates c-jun Expression

Another embodiment of a genetic construct derives

5   from the c-jun protooncogene and related genes. Ionizing
radiation regulates expression of the c-jun
protooncogene, and also of related genes c-fos and jun-β.
The protein product of c-jun contains a DNA binding
region that is shared by members of a family of

10   transcription factors. Expression level after radiation
is dose dependent. The c-jun gene encodes a component of
the AP-1 protein complex and is important in early
signaling events involved in various cellular functions.
AP-1, the product of the protooncogene c-jun recognizes

15   and binds to specific DNA sequences and stimulates
transcription of genes responsive to certain growth
factors and phorbol esters (Bohmann, et al., 1987; Angel,
et al., 1988). The product of the c-jun protooncogene
contains a highly conserved DNA binding domain shared by

20   a family of mammalian transcription factors including
jun-β, jun-D, c-fos, fos-β, fra-1 and the yeast GCN4
protein.

In addition to regulating expression of the c-jun

25   gene, c-jun transcripts are degraded
posttranscriptionally by a labile protein in irradiated
cells. Posttranscriptional regulation of the gene's
expression is described in Sherman, et al., 1990.

30   Contrary to what would be expected based on previous
DNA damage and killing rates for other agents, decreasing
the dose rate, for example, from 14.3 Gy/min to 0.67
Gy/min. was associated with increased induction of c-jun
transcripts.

35

FIG. 6. Effects of ionizing radiation on c-jun RNA
levels in human HL-60 cells. (A) Northern blot analysis
of total cellular RNA levels was performed in HL-60 cells
after treatment with 20 Gy of ionizing radiation (XRT).

5   Hybridization was performed using a ^{32}P-labeled c-jun or
actin DNA probe. (B) HL-60 cells were treated with the
indicated doses of ionizing radiation. RNA was isolated
after 3 hours and hybridizations were performed using ^{32}P-
labeled c-jun or β-actin DNA probes. The column labelled

10   HL-60 represents RNA from untreated cells.

Maximum c-jun mRNA levels were detectable after 50
Gy of ionizing radiation (FIG. 6B).

15   Similar kinetics of c-jun induction were observed in
irradiated human U-937 monocytic leukemia cells (FIG. 7A)
and in normal human AG-1522 diploid fibroblasts (FIG.
7B). Treatment of AG-1522 cells with ionizing radiation
was also associated with the appearance of a minor 3.2-kb

20   c-jun transcript.

Cell Culture. Human HL-60 promyclocytic leukemia
cells, U-937 monocytic leukemia cells (both from American
Type Culture Collection), and AG-1522 diploid foreskin

25   fibroblasts (National Institute of Aging Cell Repository,
Camden, NJ) were grown in standard fashion. Cells were
irradiated using either Philips RT 250 accelerator at 250
kV, 14 mA equipped with a 0.35-mm Cu filter or a
Gammacell 1000 (Atomic Energy of Canada, Ottawa) with a

30   ^{137}Cs source emitting at a fixed dose rate of 14.3 Gy/min
as determined by dosimetry. Control cells were exposed
to the same conditions but not irradiated.

Northern Blot Analysis. Total cellular RNA was

35   isolated as described (29). RNA (20 μg per lane) was
separated in an agarose/formaldehyde gel, transferred to

a nitrocellulose filter, and hybridized to the following
^{32}P-labeled DNA probes: (i) the 1.8-kilobase (kb)
BamHI/EcoRI c-jun cDNA (30); (ii) the 0.91-kb Sca I/Nco I
c-fos DNA consisting of exons 3 and 4 (31); (iii) the

5   1.8-kb EcoRI jun-B cDNA isolated from the p465.20 plasmid
(32); and (iv) the 2.0-kb PstI β-actin cDNA purified from
pA1 (33). The autoradiograms were scanned using an LKB
UltroScan XL laser densitometer and analyzed using the
LKB GelScan XL software package. The intensity of c-jun

10   hybridization was normalized against β-actin expression.

       Run-On Transcriptional Analysis. HL-60 cells were
treated with ionizing radiation and nuclei were isolated
after 3 hours. Newly elongated ^{32}P-labeled RNA

15   transcripts were hybridized to plasmid DNAs containing
various cloned inserts after digestion with restriction
endonulceases as follows: (i) the 2.0-kb Pst I fragment
of the chicken β-actin pA1 plasmid (positive control);
(ii) the 1.1-kb BamHI insert of the human β-globin gene

20   (negative control, ref.34); and (iii) the 1.8-kb
BamHI/EcoRI fragment of the human c-jun cDNA from the
pBluescript SK(+) plasmid. The digested DNA was run in a
1% agarose gel and transferred to nitrocellulose filters
by the method of Southern. Hybridization was performed

25   with 10^7 cpm of ^{32}P-labeled RNA per ml of hybridization
buffer for 72 h at 42·C. Autoradiography was performed
for 3 days and the autoradiograms were scanned as already
described.

30   EXAMPLE 3
       Radiation Induced Transcription of JUN and EGR1

       There was increased mRNA expression for different
classes of immediate early response to radiation genes

35   (JUN, EGR1) within 0.5 to 3 hours following cellular x-
irradiation. Preincubation with cycloheximide was

associated with superinduction of *JUN* and *EGR1* in x-
irradiated cells. Inhibition of protein kinase C (PKC)
activity by prolonged stimulation with TPA or the protein
kinase inhibitor H7 prior to irradiation attenuated the
5   increase in *EGR1* and *JUN* transcripts. These data
implicated *EGR1* and *JUN* as signal transducers during the
cellular response to radiation injury and suggested that
this effect is mediated in part by a protein kinase C
(PKC) dependent pathway.
10

JUN homodimers and *JUN/FOS* heterodimers regulate
transcription by binding to *AP1* sites in certain promoter
regions (Curran and Franza, 1988). The *JUN* and *FOS* genes
are induced following x-ray exposure in human myeloid
15   leukemia cells suggests that nuclear signal transducers
participate in the cellular response to ionizing
radiation.

EGR1 (also known as zif/268, NGFI-1, Krox-24, TIS-8)
20   (Christy, et al., 1988; Milbrant, 1987; Lemaire, et al.,
1988; Lim, et al., 1987) encodes a nuclear phosphoprotein
with a Cys_2-His_2 zinc-finger motif which is partially
homologous to the corresponding domain in the Wilms'
tumor susceptibility gene (Gessler, 1990). The *EGR1*
25   protein binds with high affinity to the DNA sequence
CGCCCCCGC in a zinc-dependent manner (Christy and
Nathans, 1989; Cao, 1990). *EGR1* represents an immediate
early gene which is induced during tissue injury and
participates in signal transduction during cellular
30   proliferation and differentiation.

The *EGR1* and *JUN* genes are rapidly and transiently
expressed in the absence of de novo protein synthesis
after ionizing radiation exposure. *EGR1* and *JUN* are most
35   likely involved in signal transduction following x-
irradiation. Down regulation of PKC by TPA and H7 is

associated with attenuation of *EGR1* and *JUN* gene
induction by ionizing radiation, implicating activation
of PKC and subsequent induction of the *EGR1* and *JUN* genes
as signaling events which initiate the mammalian cell

5   phenotypic response to ionizing radiation injury.

Control RNA from unirradiated cells demonstrated low
but detectable levels of *EGR1* and *JUN* transcripts. In
contrast, *EGR1* expression increased in a dose dependent

10   manner in irradiated cells. Levels were low but
detectable after 3 Gy and increased in a dose dependent
manner following 10 and 20 Gy. Twenty Gy was used in
experiments examining the time course of gene expression
so that transcripts were easily detectable. Cells

15   remained viable as determined by trypan dye exclusion
during this time course. A time dependent increase in
EGR1 and *JUN* mRNA levels was observed. SQ-20B cells
demonstrated coordinate increases in *EGR1* and *JUN*
expression by 30 minutes after irradiation that declined

20   to baseline within 3 hours. In contrast, *EGR1* transcript
levels were increased over basal at 3 hours while *JUN* was
increased at one hour and returned to basal at 3 hours in
AG1522. *JUN* levels were increased at 6 hours in 293
cells while *EGR1* was increased at 3 hours and returned to

25   basal levels by 6 hours.

To determine whether *EGR1* and *JUN* participated as
immediate early genes after x-irradiation, the effects of
protein synthesis inhibition by CHI were studied in cell

30   lines 293 and SQ-20B after x-ray exposure. CHI treatment
alone resulted in a low but detectable increase in *EGR1*
and *JUN* transcripts normalized to 7S. In the absence of
CHI, the level of *EGR1* and *JUN* expression returned to
baseline. In contrast, SQ-20B cells pretreated with CHI

35   demonstrated persistent elevation of *EGR1* at 3 hours and
293 cells demonstrated persistent elevation of *JUN* mRNA

at 6 hours after irradiation thus indicating
superinduction of these transcripts.

mRNA levels of transcription factors *EGR1* and *JUN*
5   increased following ionizing radiation exposure in a time
and dose dependent manner. The potential importance of
the induction of *EGR1* and *JUN* by ionizing radiation is
illustrated by the recent finding that x-ray induction of
the PDGF alpha chain stimulates proliferation of vascular
10   endothelial cells (Witte, et al., 1989). PDGF has AP-1
and *EGR1* binding domains while TNF has elements similar
to AP-1 and *EGR1* target sequences (Rorsman, et al., 1989;
Economou, et al., 1989). X-ray induction of PDGF and TNF
appears to be regulated by *EGR1* and *JUN*.

15

The following is a method used in EXAMPLE 3:

Kinase Inhibitors

20

.Cell line SQ-20B was pretreated with 1 μM TPA for 40
hours to down regulate PKC and then stimulated with TPA,
serum, or x-ray (20 Gy). Controls included x-ray without
TPA pretreatment, TPA (50 nM) without TPA pretreatment
25   and untreated cells. RNA was isolated after one hour and
hybridized to *EGR1*. SQ-20B cells were preincubated with
100 μM H7 (1-(5-isoquinolinylsulfonyl)-2-methyl
piperazine) or 100 μM HA1004 (N-[2-methyl-amino] ethyl)-
5-isoquino-linesulfonamide) Seikagaku America, Inc., St.
30   Petersberg, FL) for 30 minutes or TPA pretreatment (1 μM)
for 40 hours and followed by exposure to 20 Gy x-
irradiation. RNA was extracted one hour after
irradiation. Positive control cells treated under the
same conditions but in the absence of inhibitor also
35   received 20 Gy, while negative control cells received
neither H7 nor X-ray. RNA was extracted at one hour

after 20 Gy without inhibitor. Northern blots were
hybridized to *EGR1* or 7S. 293 cells pretreated with the
above inhibitors were irradiated, RNA was extracted after
3 hours and the Northern blot was hybridized to *JUN* and
5 7S probes.

EXAMPLE 4

Protocol for Treatment of Head and Neck Cancer
10   with X-ray Induced TNF and Therapeutic X-rays

For treatment of patients with head and neck cancer,
the following steps are followed:

15   1.   Prepare a genetic construct according to the
general scheme illustrated in FIGS. 1 or 2.

This construct comprises AP-1 as the element which
is responsive to x-rays, coupled to a sequence of DNA to
20 which the lac repressor binds, and to the gene for the
tumor necrosis factor. This construct is designated
"construct A" for purposes of this example.

2.   "Construct A" is put into a retrovirus that is
25 self-inactivating (see FIG. 3).

3.   Lymphokine activated killer (LAK) cells are
infected with the retrovirus bearing "construct A." The
cells are to be directed against the malignant cells in
30 the head and neck.

4.   The lymphocytes are infused into the patient to
be treated.

35   5.   The head and neck region is irradiated.

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CLAIMS:

1. A method for destroying, altering, or inactivating cells within a tissue, said method comprising:

5

    (a) preparing a genetic construct comprising a radiation responsive enhancer-promoter region and a region comprising at least one structural gene which is controlled by the enhancer-

10   promoter;

    (b) delivering the construct into cells in the tissue or into cells that migrate to the tissue; and

15

    (c) exposing the tissue to ionizing radiation to induce the expression of the structural gene.

20 2. The method of claim 1 wherein the radiation responsive enhancer-promoter region is derived from at least one of the following genes: c-jun, AP-1 and tumor necrosis factor.

25

 3. The method of claim 1 wherein the structural gene region comprises genes whose expression comprises at least one of the following: tumor necrosis factor, ricin, and streptokinase.

30

 4. The method of claim 1 wherein the structural gene region comprises a DNA binding domain, a repressor gene, a binding region for the repressor and a structural gene.

35

5.   The method of claim 1 wherein the ionizing radiation
is delivered at a low dose.

5   6.   The method of claim 5 wherein the dose is in the
range of 150 to 300 rads.

7.   The method of claim 6 wherein the dose is about 200
10   rads.

8.   The method of claim 1 wherein the tissue is a
disease-related tissue.
15

9.   The method of claim 8 wherein the disease-related
tissue comprises a tumor.

20

10.· The method of claim 8 wherein the disease-related
tissue comprises a blood clot.

25   11.   The method of claim 1 wherein the tissue is
characterized by a metabolic deficiency.

12.   A method of treating a disease comprising:
30

        (a) preparing a genetic construct comprising a
            promoter which is inducible by ionizing
            radiation, and at least one structural gene;

(b) delivering the genetic construct to the disease-
     related tissue so that the cells within the
     tissue incorporate the construct; and

5   (c) exposing the tissue to ionizing radiation to
             induce the expression of the gene.

     13.   The method of claim 12 wherein the genetic construct
10   comprises the c-*jun* promoter and the structural gene for
     the tumor necrosis factor.

     14.   The method of claim 12 wherein the structural gene
15   which is capable of being activated by a transcription
     factor, the expression of which is under the control of
     the radiation inducible promoter.

20   15.   The method of claim 14 wherein the structural gene
     comprises a fusion gene comprising a lac repressor, a DNA
     binding domain of a LAC repressor, a VP16 actuation
     domain, a LAC repressor binding sequence, and a reporter-
     effector gene which is activated by a lac repressor.

25

     16.   The method of claim 12 wherein the target tissue is
     a tumor and the disease to be treated is cancer.

30
     17.   The method of claim 12 wherein the target tissue is
     a blood clot and the disease to be treated is myocardial
     infarction of the myocardium, brain, lung or other
     tissues.

35

18.   A method of causing the expression within a host
tissue of a structural gene, said method comprising:

(a) delivering *in vitro* the vector comprising a
     radiation responsive enhancer-promoter region
     and a structural gene to cells derived from the
     host tissue;

(b) reintroducing the cells from the host tissue to
     the host; and

(c) activating the genes within the vector by
     exposure of the tissue to radiation.

19.   The method of claim 18 wherein the radiation
exposure is delivered at a dose of about 200 rads.

20.   A method for destroying, altering, or inactivating
cells, comprising:

(a) incorporating into the cells a genetic construct
     which comprises genes whose expression is
     inducible by ionizing radiation and whose
     products are capable of destroying, altering or
     inactivating the cells; and

(b) exposing the construct containing cells to
     ionizing radiation to induce the expression of
     genes in the genetic construct.

21.   A genetic construct comprising a promoter which is
inducible by ionizing radiation and a structural gene.

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22. The genetic construct of claim 21 wherein the structural gene is a mammalian gene.

5

23. The genetic construct of claim 21 further defined as having the inducible promoter 5' to the structural gene.

10   24. The genetic construct of claim 21 comprising the promoter for the tumor necrosis factor and the structural gene for the tumor necrosis factor.

15   25. The genetic construct of claim 21 wherein the structural gene comprises a plant toxin gene.

26. A vector comprising a radiation responsive enhancer-
20   promoter region and a structural gene.

27. A vector comprising a radiation responsive promoter which controls the expression of a transcription factor,
25   said transcription factor being capable of activating a genetic construct comprising a structural gene.

28. A method for controlling structural gene expression,
30   said method comprising:

(a) preparing a genetic construct comprising the
        structural gene to be expressed and a mammalian
        genetic promoter-enhancer region which is
35   inducible by ionizing radiation;

-45-

   (b) exposing the genetic construct to ionizing
       radiation at a dose sufficient to induce
       expression of the structural gene.

5

   29.   The method of claim 28 wherein the promoter-enhancer
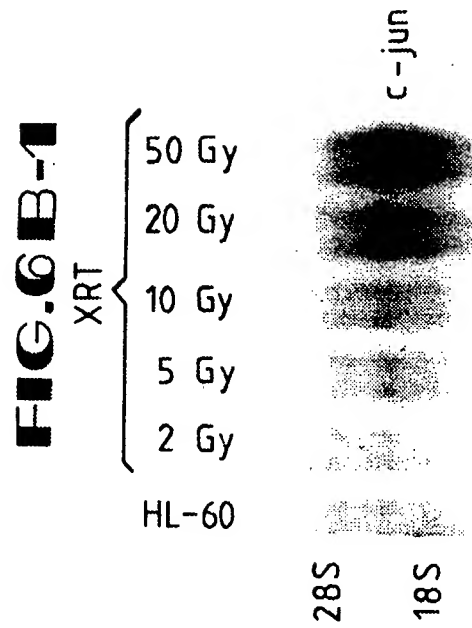   region is derived from the genes coding for the *c-jun* or
   TNF-α proteins.

FIG.1

FIG.2A

FIG.2B

FIG.3

3/8

FIG.11

FIG.4A

FIG.4B

FIG.5C

FIG.5B

FIG.5A

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FIG.6B-1

FIG.6B-2

FIG.6A-1

FIG.6A-2

FIG.7A

FIG.7B

FIG.8
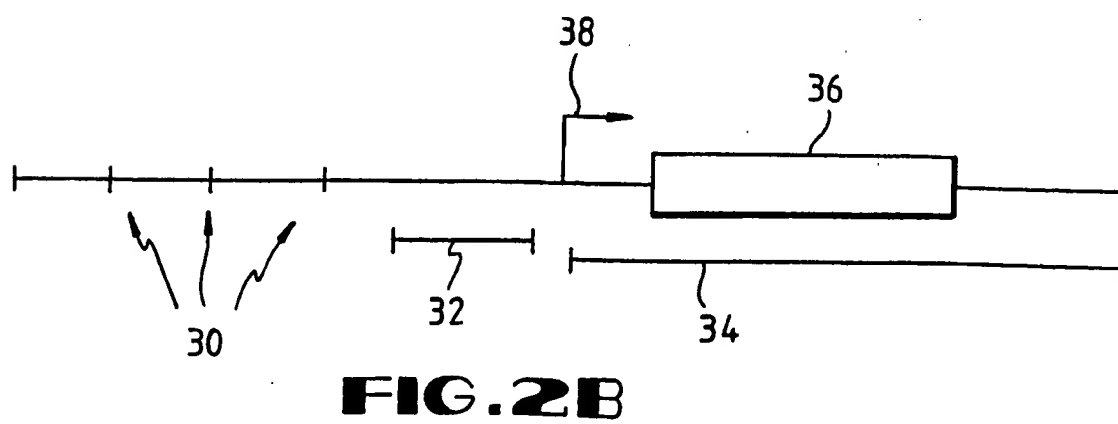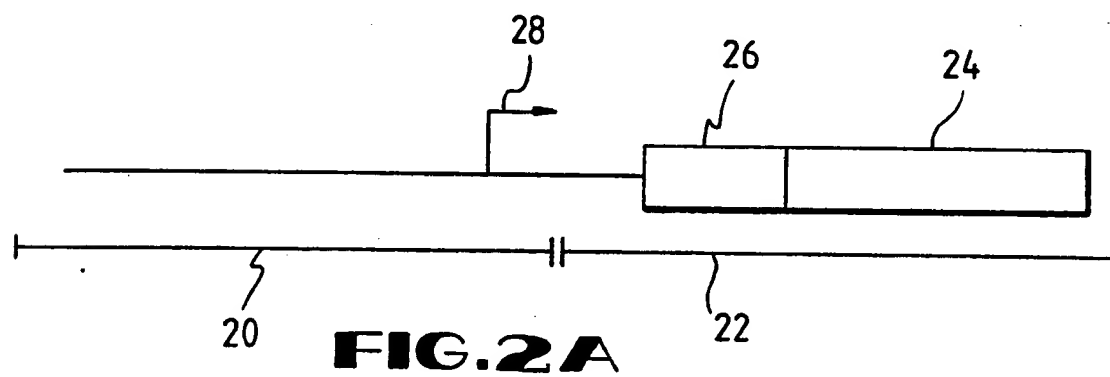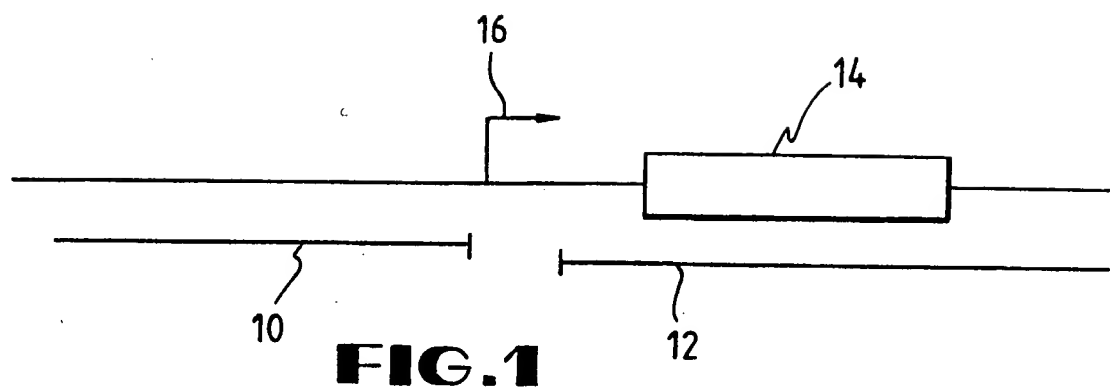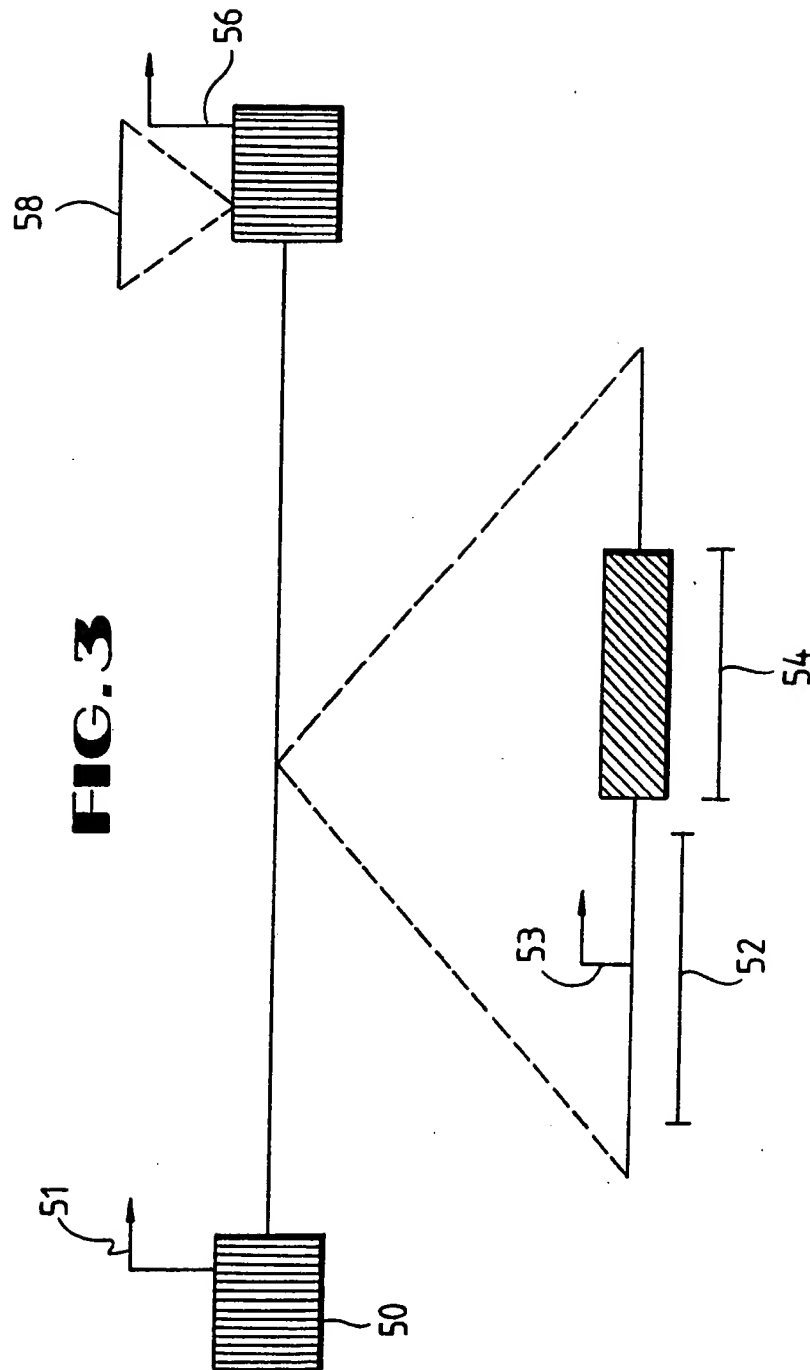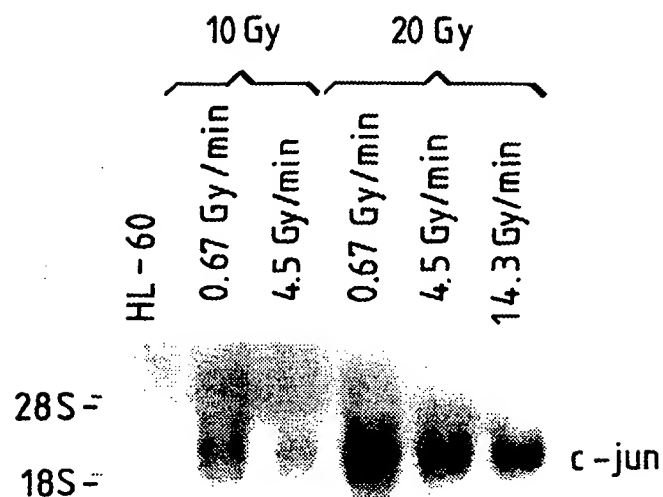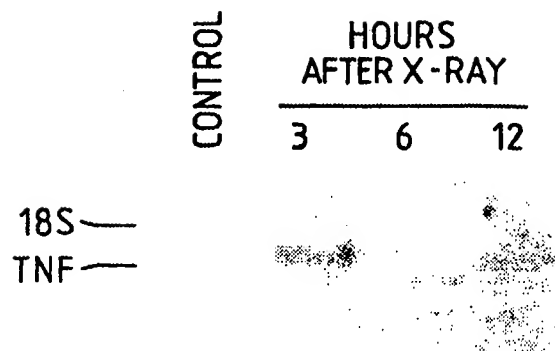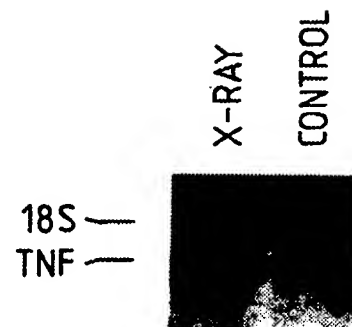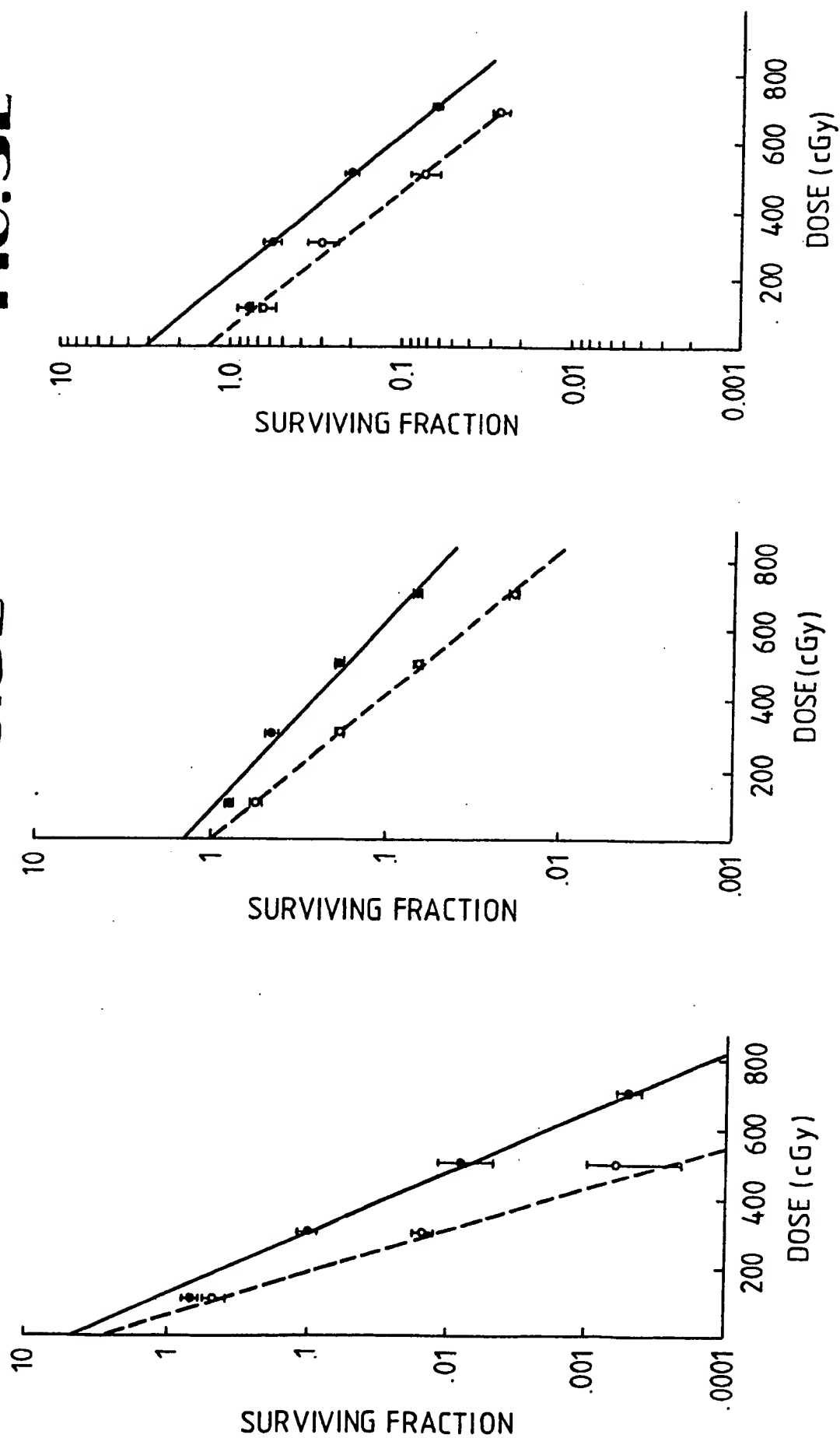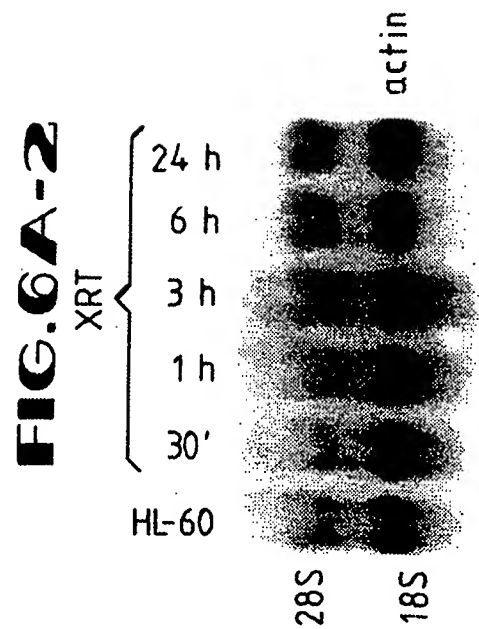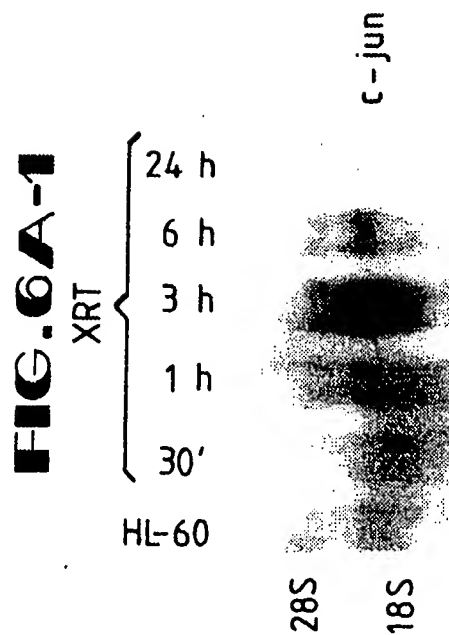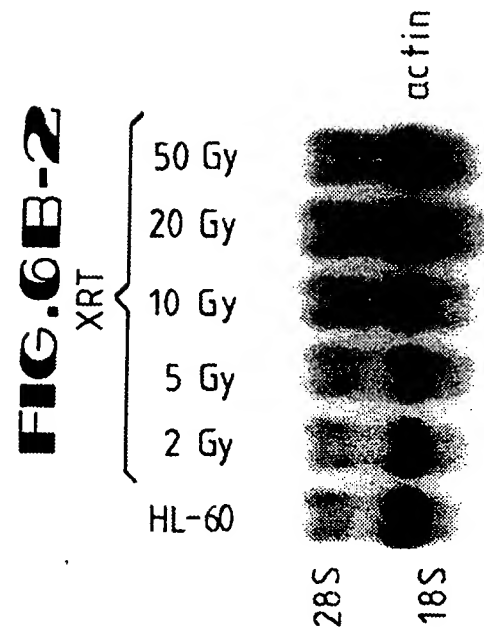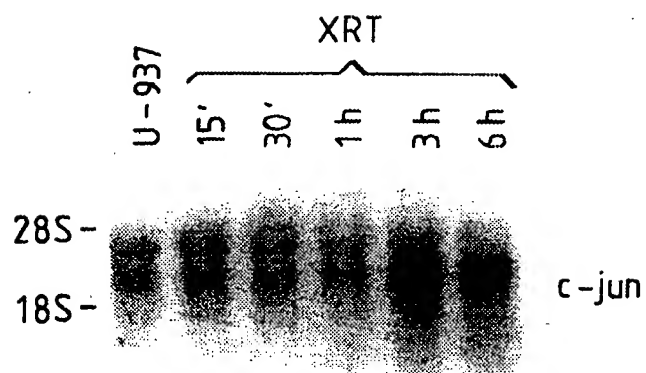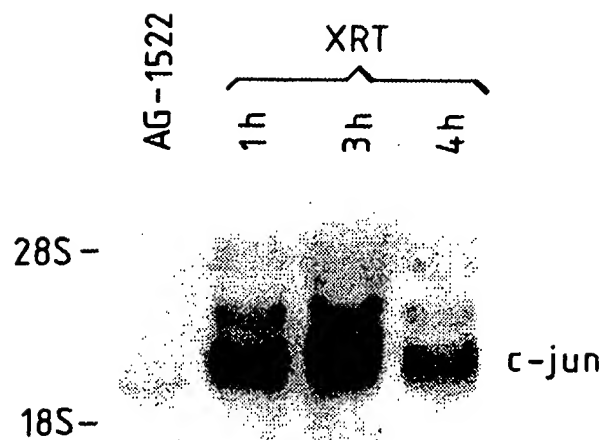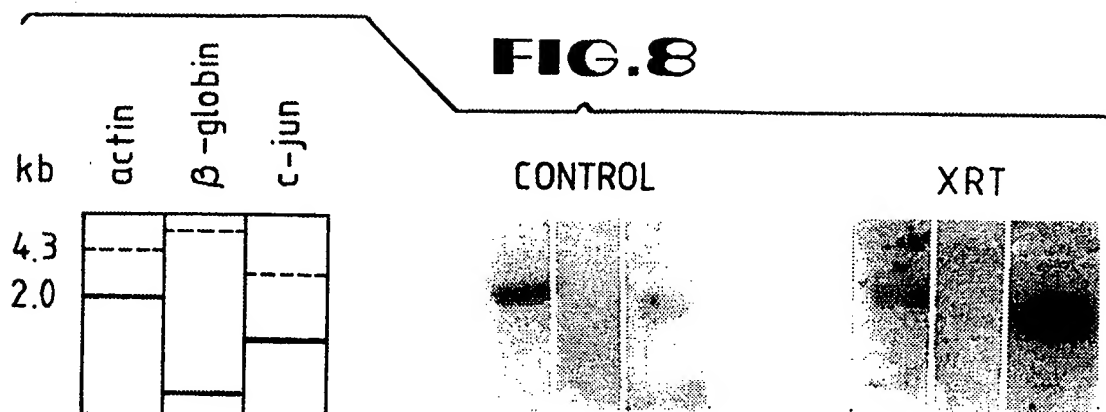
FIG.9A
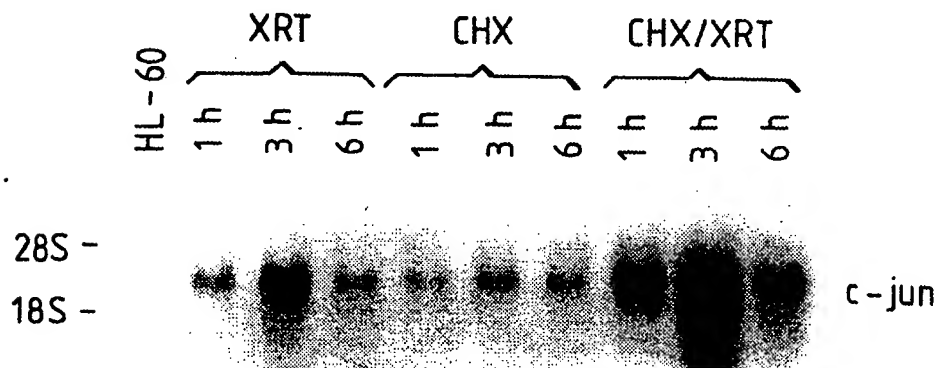
FIG.9B
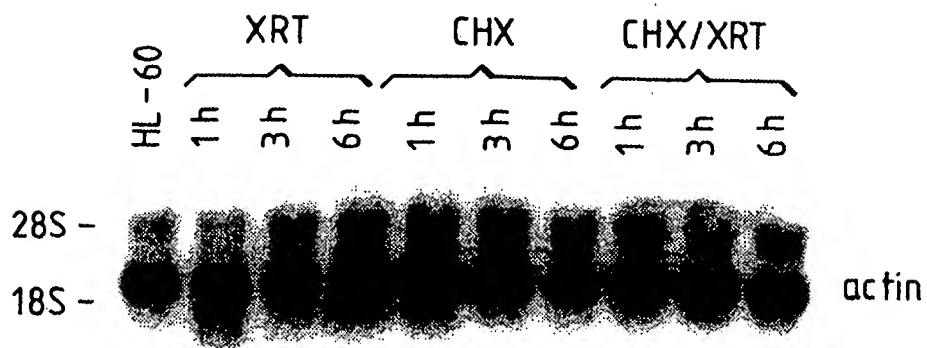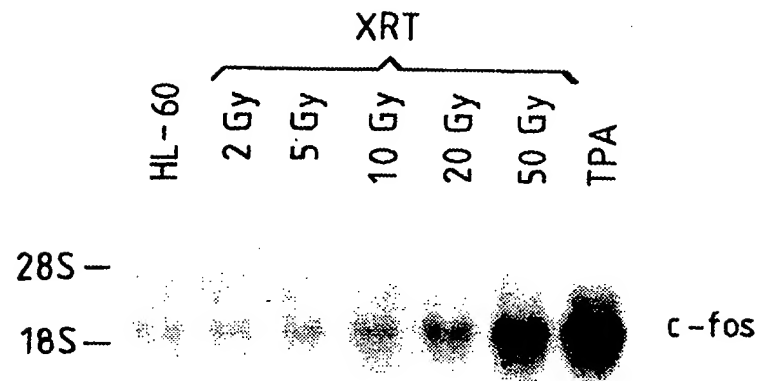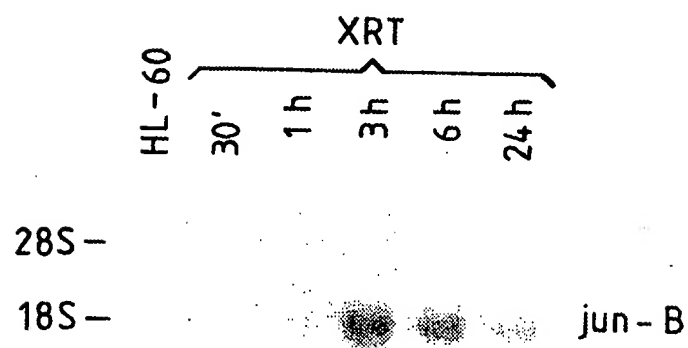
FIG.10A

FIG.10B

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 43/00; C12Q 1/68; C07H 15/12; A01N 43/04
US CL : 424/1.1; 435/6; 536/27; 514/44; 935/6, 55, 62

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
US	424/1.1; 435/6; 536/27; 514/44; 935/6, 55, 62

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category[*]	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
X	Int. J. Cancer, Volume 42, issued 1988, G. Sersa et al., "Anti-Tumor Effects of Tumor Necrosis Factor alone or combined with Radiotherapy", pages 129-134, see the entire document.	1-29
X	Cancer Research, Volume 49, Number 18, issued 15 September 1989, L. Witte, et al., "Effects of irradiation on the release of growth factors from cultured bovine, porcine, and human endothelial cells", pages 5066-5072, see abstract no. 07052277.	1-29
X	Proceedings of the National Academy of Science (USA), Volume 87, issued August 1990, M.L. Sherman, et al., "Ionizing radiation regulates expression of the c-jun protooncogene", pages 5663-5666, see entire document.	1-29
X	Proceedings of the National Academy of Science (USA), Volume 86, issued December 1989, D.E. Hallman, et al., "Increased tumor necrosis factor amRNA pages 10104-10107", see entire document.	1-29

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
20 March 1992	02 APR 1992
International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	Bradley Sisson

Form PCT/ISA/210 (second sheet)(May 1986) ß